Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 43 (2008) 1536–1542
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Short communication

Synthesis, characterization, antifungal and anti-HIV activities of metal(II) complexes of 4,6-di-*tert*-butyl-3-[(2-hydroxyethyl)thio]benzene-1,2-diol

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Received 17 July 2007; received in revised form 20 September 2007; accepted 28 September 2007
Available online 5 October 2007

Abstract

Co(II) and Ni(II) complexes with 4,6-di-*tert*-butyl-3-[(2-hydroxyethyl)thio]benzene-1,2-diol (L) have been synthesized and characterized by means of elemental analysis, TG/DTA, FT-IR, ESR, UV–vis, XRD, magnetic susceptibility, cyclic voltammetry and conductance measurements. According to the data obtained the organic compound acts as a bidentate *O*,*S*-coordinated ligand and yields Co(II) and Ni(II) complexes of the stoichiometry ML_2 which is characterized by square planar geometry. Antifungal and anti-HIV activities of the ligand and its metal(II) complexes were found to decrease in the sequence $CuL_2 > CoL_2 \sim NiL_2 > HL$, along with their reducing ability (determined electrochemically).

Keywords: Metal(II) complexes; Sterically hindered *o*-diphenol derivative; Antifungal activities; Anti-HIV activity

1. Introduction

The new medical technologies introduced into clinical practice (transplantation of organs and tissues, immunosuppressive therapy), the HIV infection pandemic and wide employment of antibacterial pharmaceuticals resulting in an increased number of immunocompromised patients running a high risk of developing opportunistic infections like fungal, tuberculosis, viral and neoplastic diseases has become more and more important problem of the modern medicine [1,2]. Invasive mycoses are the most common opportunistic infections in patients with AIDS. In this connection it is of interest to seek for bioactive compounds, exhibiting both anti-HIV and antifungal activities. Despite the fact that it is *Candida* spp. and *Aspergillus* spp. that remain the main causative pathogens, the number of cases of systemic fungal infections due to strains of *Fusarium* spp., *Scedosporium* spp., *Mucor* spp. and others, resistant to the most widely used antifungal polyene and azole drugs, is increasing [3–5]. In this connection a search for possible alternative antifungal agents by widening existing classes and producing new ones is a pressing task. Due to the possibility of their antifungal activity mode being different, metal complexes might provide the basis for novel antifungal agents, which could have potential applications as pharmaceuticals. Reports have appeared in the literature highlighting the fungicidal activity of transition metal complexes with 1,10-phenanthroline [6–9], thiosemicarbazones [10–12], carboxylates [13], dithiocarbamates [14] and thiourea derivatives [15–18]. Thus, the synthesis and characterization of metal complexes with organic bioactive ligands, in particular, of those with derivatives of sterically hindered

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doi:10.1016/j.ejmech.2007.09.024

o-diphenols (SHD), is one of the promising areas of the search for potential chemotherapeutic agents. An effective bioantioxidant has been found among them [19], moreover, a sulphurous derivative thereof exhibits a high antiviral activity [20,21]. Although derivatives of SHD exhibit interesting biological properties [22,23] and a great versatility as noninnocent ligands [24–29], still there is virtually no pharmacological or coordinative information about their sulphurous derivatives. In this connection it is of interest to study various aspects of metal(II) coordination chemistry of these organic compounds and the biological activity of their metal complexes. Recently we have reported the synthesis, characterization and antimicrobial activities of some SHD derivatives as well as their Cu(II) complexes [30,31]. Since our earlier work had revealed that Cu(II) complexation leads to enhancement of the antimicrobial activity of these ligands, we were motivated to explore whether there is a similar trend in the case of other transition metal(II) complexes of these organic compounds. We report here the synthesis and characterization of Co(II), Ni(II) complexes with one of the sulphurous derivatives of SHD, that is 4,6-di-*tert*-butyl-3-[(2-hydroxyethyl)thio]benzene-1,2-diol (L), in order to compare its coordinative behaviour in relation to Co(II), Ni(II) ions with the results obtained before for its Cu(II) complex [30,31] and to assess the influence of complexation on antifungal and anti-HIV activities. The said *o*-diphenol derivative was chosen as the ligand to synthesize metal(II) complexes because it is relatively stable to oxidation, and its Cu(II) complex had previously demonstrated a promising activity against some fungi strains.

It should be noted that the effect of some transition metal complexes on the structure of fungal and mammalian cell organelles has been studied, and the general conclusion is that they have the potential to damage mitochondrial function and to uncouple respiration [9]. In this connection it may be expected that metal complexes which are able to participate in redox processes and affect the electron-transport cell systems will be promising in the search for antifungal agents. This encouraged us to examine redox properties of the ligand and its metal(II) complexes by cyclic voltammetry to find out whether there is a correlation between antifungal activities of the compounds under study and their redox properties.

2. Chemistry

2.1. Materials and methods

Chemicals were purchased from commercial sources and were used without further purification. The SHD derivative was prepared according to Refs. [20,21,32]. Elemental analyses were carried out according to the standard methods by Microanalytical Laboratory, Bioorganic Chemistry Institute, National Academy of Sciences, Belarus. Metal and sulfur determination was carried out using an atomic emission spectrometer with an inductively coupled plasma excitation source (Spectroflame Modula). Infrared spectra of solids (the ligand and metal(II) complexes) were recorded by a Spectrum 1000 spectrophotometer in the wavelength range 4000–400 cm^{-1} at room temperature, using nujol mulls of solids and polyethylene windows. Thermal analysis was performed with a derivatograph OD-103 MOM. TG/DTA measurements were run in the air between 20 and 450 °C (5 °C min^{-1}). XRD studies were made with an HZG 4A diffractometer (Co Kα or Cu Kα radiation, MnO_2-filter). ESR spectra of polycrystalline samples were measured on ERS-220 X-band spectrometer (9.45 GHz) at room temperature and at 77 K, using 100-kHz field modulation; *g* factors were quoted relative to the standard marker DPPH ($g = 2.0036$). Magnetic susceptibility measurements of solids were determined by a Gouy balance at room temperature using $Hg[Co(SCN)_4]$ as a calibrant. UV–vis spectra were recorded on a SPECORD M500 spectrophotometer. The molar conductance of 10^{-3} mol L^{-1} solutions of the metal(II) complexes in acetonitrile was measured at 20 °C using a TESLA BMS91 conductometer (cell constant 1.0). Electrochemical measurements were performed under dry nitrogen in a three-electrode two-compartment electrochemical cell using a glassy-carbon (GC) working electrode, Pt auxiliary electrode and Ag|AgCl|0.1 mol L^{-1} $(C_2H_5)_4NCl$ reference electrode. The supporting electrolyte was 0.1 mol L^{-1} $(C_2H_5)_4NClO_4$. The Ag|AgCl|0.1 mol L^{-1} $(C_2H_5)_4NCl$ reference electrode was calibrated with the ferricinium|ferrocene redox couple, located at $E_{1/2} = +0.52$ V. Acetonitrile was used as a solvent.

2.2. Synthesis of the metal(II) complexes with 4,6-di (tert-butyl)-3-(2-hydroxyethylsulfanyl)-1,2-benzenediol

Based on our previous findings [31], the conditions were created to purposefully provide the preferential formation of the complex with molar ratio M(II):L = 1:2 (M = Co(II), Ni(II)): a solution of metal(II) salt was added in small portions to the ligand solution under continuous stirring, so that the complexation always took place with the excess ligand present. The preparation of metal complexes follows a common procedure. A solution of 0.050 mmol $M(CH_3COO)_2 \cdot 4H_2O$ in 10 mL of water was added dropwise to a colorless solution of 0.100 mmol of the ligand dissolved in 10 mL of ethanol (molar ratio M(II):L = 1:2). As these ligands can be readily oxidized by oxygen, argon was bubbled through the solutions (pH ≤ 6) during the synthesis to ensure the absence of oxygen. Colored precipitates of metal(II) complexes formed instantaneously. After 1.5 h of stirring, they were collected on membrane filters (JG 0.2 μm), washed with ethanol and water, and dried *in vacuo* (yield > 70%).

2.2.1. CuL_2 complex

For elemental analyses data of CuL_2 see Ref. [36].

2.2.2. CoL_2 complex

Yellow. Anal. Calc. for $C_{32}H_{50}S_2O_6Co$: C, 58.81; H, 7.71; S, 9.82; Co, 9.02. Found: C, 58.73; H, 7.65; S, 9.74; Co, 8.94.

2.2.3. NiL_2 complex

Green. Anal. Calc. for $C_{32}H_{50}S_2O_6Ni$: C, 58.83; H, 7.71; S, 9.82; Ni, 8.99. Found: C, 58.74; H, 7.61; S, 9.73; Ni, 8.90.

2.3. Physico-chemical characterization

2.3.1. CuL_2 complex

For physical and spectral characteristics of CuL_2 see Ref. [36].

2.3.2. CoL_2 complex

Molar conductivity (in acetonitrile): $\Lambda_{mol} = 3.1\ \Omega^{-1}\,cm^2\,mol^{-1}$. TG/DTA data: no weight loss was observed until decomposition which began at about 180 °C, with exothermic peaks at 345 °C (without any noticeable weight loss) and at 390 °C, ultimately leaving CoO as the residue. The maximal weight loss of 87.4% corresponds to the loss of two ligand molecules in the CoL_2 complex (Calc. 88.6%). Prominent IR bands (nujol mull) (cm^{-1}): 532w, 581w ν(Co–O), 340w ν(Co–S), 634m ν(C–S), 1056m ν(C–O), 3338w ν(–OH), 1544w, 1600w ν(C=C). UV–vis data (acetonitrile) (λ_{max}, nm): 510sh, 400, 320, 255, 225.

2.3.3. NiL_2 complex

Molar conductivity (in acetonitrile): $\Lambda_{mol} = 2.2\ \Omega^{-1}\,cm^2\,mol^{-1}$. TG/DTA data: no weight loss was observed until decomposition which began at about 210 °C, with exothermic peaks at 230 °C (without any noticeable weight loss) and at 323 °C, ultimately leaving NiO as the residue. The maximal weight loss of 87.5% corresponds to the loss of two ligand molecules in the NiL_2 complex (Calc. 88.6%). Prominent IR bands (nujol mull) (cm^{-1}): 532w, 578w ν(Ni–O), 345w ν(Ni–S), 638m, ν(C–S), 1012m ν(C–O), 3425m ν(–OH), 1543w, 1591w ν(C=C). UV–vis data (acetonitrile) (λ_{max}, nm): 440sh, 390, 315, 255, 225.

3. Pharmacology

3.1. Antifungal assays

Antifungal activities of the compounds were tested against the following test microorganisms (the collection of Department of Microbiology, Belarusian State University): yeasts (*Pichia pastoris*, *Lypomyces lipofer*, *Saccharomyces cerevisiae*, *Cryptococcus laurentiive*, *Candida utilis*, *Candida boidinii*) and fungi (*Aspergillus niger*, *Fusarium* spp., *Mucor* spp., *Penicillium lividum*, *Botrytis cinerea*, *Alternaria alternata*, *Sclerotinia sclerotiorum*, *Monilia* spp.).

Bioactivities of the compounds against yeasts were estimated by a minimum inhibitory concentration (MIC, $\mu g\,mL^{-1}$) as described elsewhere [33]. Each compound was dissolved in dimethyl sulfoxide and added to Sabouraud medium in order to obtain concentrations from 200 to 3125 $\mu g\,mL^{-1}$. A two-fold serial dilution was used. In every case MIC was determined as the lowest concentration of the compound under study, which inhibits the visible yeasts growth, compared with the control system in which the microorganisms were grown in the absence of any test compound. The absence of yeast growth after an incubation period of 48 h at 25 °C was taken as a criterion of effectiveness.

Antifungal activities of the compounds against moulds were checked by the agar plate technique reported previously [33]. Compounds were dissolved in dimethyl sulfoxide and diluted in potato dextrose agar medium to yield working solutions of the test compounds with the concentration of 100 $\mu g\,mL^{-1}$. The mixtures were poured on glass Petri dishes. Once the medium had congealed, the plates were inoculated with a small piece of micelial block of each test fungus cut out from the outer margin of the culture grown on a basal medium. The inoculated plates were incubated at 25 °C, whereupon the linear growth of the fungal colony was measured in two directions at right angles to each other after 72 h, and the average of three replicates was taken as the diameter of the colony in millimeters. The concentration of dimethyl sulfoxide in the medium was 1% and did not affect the fungal growth. The results were confirmed in three independent experiments. The degree of inhibition of radial growth (RI) was calculated as follows [34]: $RI = 100(C - T)/C$ (%), where T is the mean value of the diameter of the fungal colonies in the presence of a given concentration of each compound, and C is the mean value of the diameter of the fungal colonies in the absence of the same compound, measured under the same conditions.

3.2. Anti-HIV assays

The assay involved the killing of T4 lymphocytes by HIV. T4 lymphocytes (CEM.SS and MT-4 cell lines) were exposed to HIV and treated with the synthesized compounds, dissolved in ethanol (96%); the initial concentration of the stock solutions was 5 mg mL. The CEM cells were grown in RPMI-1640 medium. The virus stocks were stored at −196 °C until used. The tests were carried out according to the therapeutic pattern (the virus was introduced into a cell suspension immediately after the compounds). The results were registered in 3–4 days. The tests were carried out using three procedures: the tetrazolium-based colorimetric assay (MTT) [35], the trypan blue dye exclusion assay (TBDE) [36], the indirect immunofluorescence assay (IIF) [37].

4. Results and discussion

The solid products resulting from the interaction of metal(II) ions with the ligand were well characterized by means of elemental analysis, TG/DTA, FT-IR, UV–vis, ESR, magnetic susceptibility and conductance measurements. These complexes were insoluble in water, ethanol, diethyl ether, nitromethane and chloroform, but they were soluble in acetonitrile and dimethyl sulfoxide. The conductivity data indicate their being essentially non-electrolytes in acetonitrile [38], and suggest that the two bidentate ligands may be coordinated to metal(II) ions as uninegatively charged ligands.

The elemental analyses data for the metal(II) complexes are in agreement with the general formula ML_2. Thermal analysis in air flow with identification of the final products by X-ray powder diffraction has shown all the complexes to be anhydrous and unsolvated, because their DTA curves lack any

endothermic peaks over a wide range from 60 to 150 °C. The agreement between the experimental and theoretical weight losses for the above processes confirms the above-mentioned general formula of the metal complexes.

All the complexes were characterized by X-ray patterns of their own, differing significantly from that of the ligand. However, a full structural analysis could not be performed because no single crystals suitable for X-ray diffraction studies were obtained. It has been reported that some compounds, in particular metal complexes with sterically hindered ligands, were not apt to form monocrystals [39,40]. Because of the well-known difficulties in direct X-ray investigations of these complexes, the geometrical arrangement of the ligating atoms in the metal complexes has been investigated by several spectroscopic and magnetochemical methods.

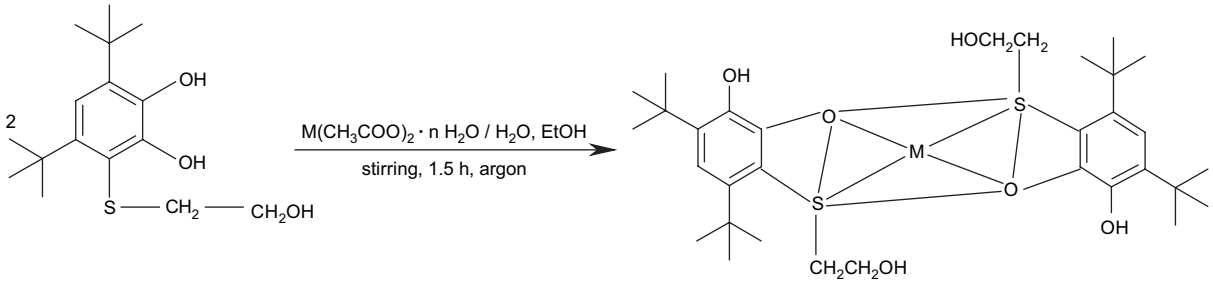
To specify the coordination cores in the Co(II) and Ni(II) complexes, we used IR spectroscopy. In the spectrum of the ligand there are two bands in the range from 3300 to 3540 cm^{-1}, indicating the presence of intermolecular hydrogen bonds involving hydroxyls [41]. As for the spectra of the metal(II) complexes, there is only one broad band in that region, which allows one to suggest that the ligand coordinates in its singly deprotonated form. The bands at 1200–1060 cm^{-1} in the spectrum of the ligand, assigned to the vibrations of C–O bond, are shifted towards lower frequencies in the spectra of metal(II) complexes, indicating metal(II) being coordinated with hydroxyl of the ligand. The shift of the bands at 850–680 cm^{-1} (assigned to C–S bond vibrations) to the low-frequency region in the spectra of metal(II) complexes suggests that the sulfur is involved in the complexation. The sensible changes in the frequencies of ν(C=C) vibrations of aromatic ring in the spectra of metal complexes compared to those of the ligand (1604 and 1561 cm^{-1}) also are evidence in favour of the coordination bond formation [42]. It should be noted that in the spectra of all the complexes under study there are new bands in the region of 470–580 cm^{-1} which may be assigned to the stretching vibrations of M–O bond. M–S stretching vibration frequencies are registered in the region 340–350 cm^{-1}.

The electronic absorption spectrum of the ligand in acetonitrile shows characteristic intraligand transition bands at 225, 255 and 295 nm, the latter disappearing upon complexation. In the spectra of the CoL_2 and NiL_2 complexes the absorption maxima at 225 and 255 nm belong to intraligand transitions. The absorption maxima appearing in these spectra at 315–320 nm and 390–400 nm are indicative of the ligand-to-metal(II) charge transfer transitions, respectively, $S \rightarrow M^{II}$ and $O_{phenolate} \rightarrow M^{II}$ [43]. The absorption band attributable to d–d transition in the spectrum of the CoL_2 complex is observed as a broad shoulder centered at about 510 nm, and in that of NiL_2 the shoulder is at 440 nm. This sort of absorption is due to the square planar MS_2O_2 chromophore [43]. These coordination cores agree with the data obtained by physico-chemical methods for the Cu(II) complex investigated previously [31], where the copper site has square planar stereochemistry.

The effective magnetic moment μ_{eff} of the Co(II) complex is 2.3 BM, which indicates that the complex has square planar geometry [44]. The value of μ_{eff} of the Ni(II) complex is zero and is also indicative of square planar configuration. No signal of stabilized radicals present in ESR spectra, as well as the ν(C=O) stretching vibrations lacking in IR spectra of metal(II) complexes, respectively, in the ranges of 1400–1500 cm^{-1}, confirm the phenolate character of the ligands.

In the light of the spectral data, magnetic moment and analytical results the mode of bonding in the metal(II) complexes can be represented as shown below (Scheme 1).

Electrochemical data obtained in de-aerated acetonitrile solution of the ligand and metal(II) complexes are presented in Fig. 1. It is known [26–29] that diphenol derivatives readily undergo electrochemical oxidation to give respective semiquinones and benzoquinones. Besides, there is a possibility of oxidation of sulfur atom in the side chain of the ligand. Cyclic voltammogram of the ligand at positive potential shows an anodic peak at 1.45 V with a shoulder (may be two overlapping peaks), another one 1.77 V, and the third anodic peak at 2.1 V on the background of anodic current growing (Fig. 1a, *solid line*). On the reverse scan there is a small cathodic peak at 0.45 V and a wave at −0.5 to −1.5 V. No prominent cathodic processes are observed on cathodic polarization from the open circuit potential down to −1.8 V (Fig. 1a, *dashed line*). On anodic polarization of GC electrode in CuL_2 complex solution an anodic wave is registered on voltammograms from the open circuit potential (70 mV) to 0.9 V, with a respective cathodic peak about 0 V on the reverse scan. Then three peaks are observed (at 1.4, 1.75 and 2.1 V) similar to those observed for the ligand (Fig. 1b, *solid line*). Cathodic peaks at 0.45 and −0.7 V are observed on the reverse scan. On cathodic polarization there is a more or less smooth growth of cathodic current, and a double anodic peak is registered on the reverse scan (two overlapping peaks) at −0.19 to −0.1 V (Fig. 1b, *dashed line*). Redox properties of CoL_2 and NiL_2 complexes are



Scheme 1.

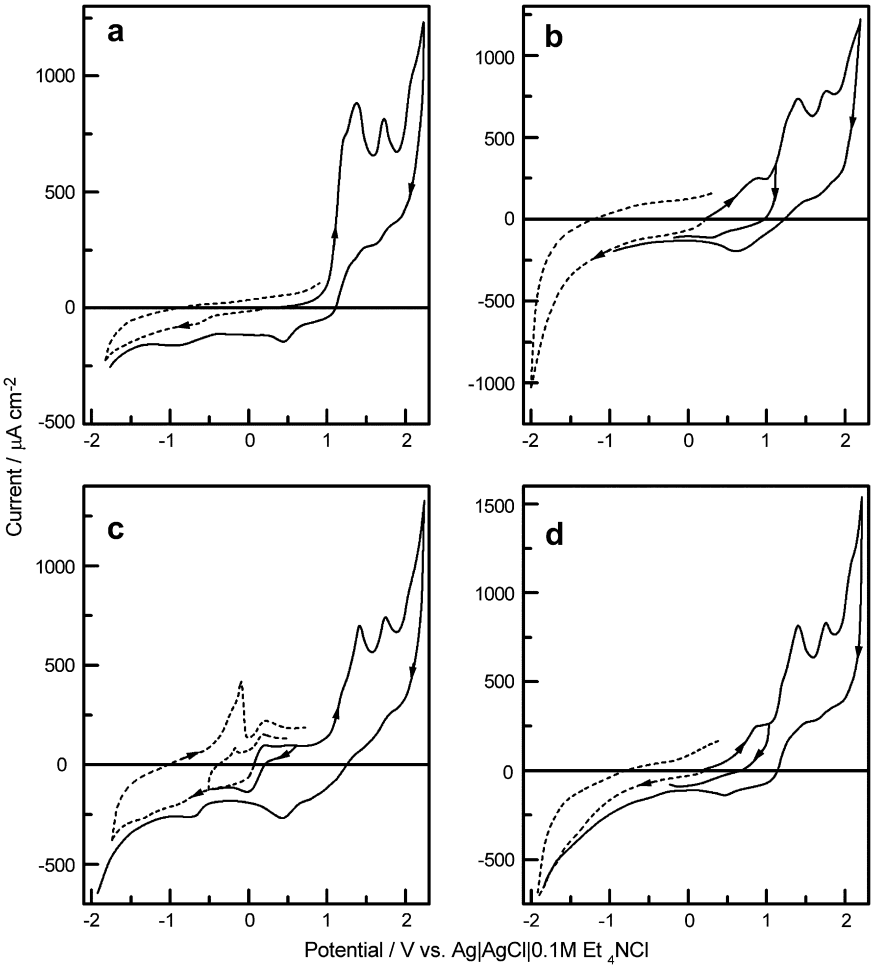


Fig. 1. Voltammograms (50 mV s^{-1}) of the ligand (1.36 mmol L^{-1}) (a), CuL_2 (0.68 mmol L^{-1}) (b), CoL_2 (0.68 mmol L^{-1}) (c) and NiL_2 (0.68 mmol L^{-1}) (d) in 0.1 mol L^{-1} $(C_2H_5)_4NClO_4$ acetonitrile solution on glassy-carbon (GC) electrode. *Solid line* − polarization in the anodic direction from the open circuit potential; *dashed line* − polarization in the cathodic direction from the open circuit potential.

similar. On anodic polarization in voltammograms there is an anodic peak at 0.87 V and three anodic peaks at 1.4, 1.75 and 2.1 V (Fig. 1c and d, *solid line*), similar to those of the ligand oxidizing. On the reverse scan a small cathodic peak is registered on voltammograms at 0.5 V. On cathodic polarization no cathodic peaks are observed down to −2 V (Fig. 1c and d, *dashed line*). Anodic waves from the open circuit potential to 0.9 V observed for the metal complexes could be assigned to M(II)→M(III) oxidation. The other peaks are related to ligand oxidation.

These findings show that the processes of oxidation for the metal complexes begin at potentials much more cathodic than those for the ligand. It is CuL_2 complex that is characterized by the most cathodic potential in these processes, with CoL_2 and NiL_2 complexes ranking next, their redox properties being virtually the same, while redox processes for the ligand begin at much more anodic potentials (Fig. 1).

Antifungal activities of the ligand and metal(II) complexes (M = Co(II), Ni(II), Cu(II))were tested against the test microorganisms: yeasts, moulds and common plant pathogens

Table 1
Antimicrobial activities of the compounds tested against yeasts (MIC, μg mL^{-1})

Compound	*Cryptococcus laurentive*	*Lypomyces lipofer*	*Pichia pastoris*	*Candida boidinii*	*Candida utilis*	*Saccharomyces cerevisiae*
L	25	50	25	>200	>200	50
CuL_2	6.25	6.25	6.25	12.5	6.25	12.5
CoL_2	25	25	25	50	25	25
NiL_2	12.5	25	12.5	50	12.5	12.5
$Cu(CH_3COO)_2$	>200	>200	>200	>200	>200	>200
$Co(CH_3COO)_2$	>200	>200	>200	>200	>200	>200
$Ni(CH_3COO)_2$	>200	>200	>200	>200	>200	>200
Nistatin	6.25	12.5	6.25	6.25	3.125	6.25

Table 2
Antifungal activities of the free ligand and its metal(II) complexes expressed as radial inhibition of mycelial growth (RI, %)

Compound	RI (%)							
	Alternaria alternata	*Sclerotinia sclerotiorum*	*Monilia* spp.	*Aspergillus niger*	*Fusarium* spp.	*Mucor* spp.	*Penicillium lividum*	*Botrytis cinerea*
L	50	60	60	50[a]	60[a]	25[a]	50[a]	65[a]
CuL_2	70	85	100	75[a]	95[a]	50[a]	80[a]	100[a]
CoL_2	65	100	85	60	90	40	65	80
NiL_2	60	100	85	60	90	25	70	80
$Cu(CH_3COO)_2$	0	0	0	0	0	0	0	0
$Co(CH_3COO)_2$	0	0	0	0	0	0	0	0
$Ni(CH_3COO)_2$	0	0	0	0	0	0	0	0
Nistatin	40	50	40	70	90	50	90	60
Terbinafine	50	50	50	100	80	50	60	60

[a] Ref. [31].

(Tables 1 and 2). For the ligand and its CuL_2 complex, continuing our previous study [31], we have expanded the spectrum of fungi, and it was for the first time that the antifungal activities of CoL_2, and NiL_2 complexes were investigated. Commonly used antifungal drugs Nystatin and Terbinafine were tested as positive controls. The effect of the starting metal(II) acetates is also reported.

Inhibition values of the free ligand and their complexes CuL_2, CoL_2, and NiL_2 against yeasts were estimated by a minimum inhibitory concentration (MIC, $\mu g\,mL^{-1}$), listed in Table 1. The activities of the metal complexes against most of the yeast strains are higher than that of the ligand. Of the metal complexes, CuL_2 proved to be the most active one, suppressing the growth of the most of the strains at MIC = 6.25 $\mu g\,mL^{-1}$. None of the starting metal salts acts against yeasts up to the dose of 200 $\mu g\,mL^{-1}$.

Inhibitory effects of the free ligand and their metal(II) complexes upon moulds and common plant pathogens are presented in Table 2. The ligand was found to be less active than the metal(II) complexes against all the fungi tested. The high activity of the metal(II) complexes under study against *Sclerotinia sclerotiorum*, *Monilia* spp., *Fusarium* spp. and *Botrytis cinerea* (RI ranging from 80 to 100%) is worth to be noticed.

Table 3
Anti-HIV activities for the ligand and their metal(II) complexes

Compound	MTC[a] ($\mu g\,mL^{-1}$)	MTC/EC_{50}[b]		
		MTT	TBDE	IIF
L	4.0	1.09	1.74	1.01
CuL_2	9.0	8.36	5.14	2.49
CoL_2	13.5	4.07	3.57	3.91
NiL_2	14.0	2.86	2.78	N.a.[c]
$Cu(CH_3COO)_2$	1.5	N.a.	N.a.	N.a.
$Co(CH_3COO)_2$	8.0	N.a.	N.a.	N.a.
$Ni(CH_3COO)_2$	3.0	N.a.	N.a.	N.a.
AZT	4.5	28.5	22.5	22.5

[a] MTC, maximal tolerable concentration of the compound, that is the highest concentration of a substance in an environmental medium that doesn't cause death of test organisms.

[b] $MTC/EC_{50} < 2$ is indicative of a low activity; $MTC/EC_{50} = 2–8$ – medium activity; $MTC/EC_{50} > 8$ – high activity.

[c] N.a., no anti-HIV activity.

When evaluating the activity of the metal complexes against several fungi it can be noted that for the most part they effectively inhibit mycelial growth ($RI \geq 70\%$)and thus may be considered as potential antifungal agents, particularly when their activity is comparable with or higher than the inhibiting effect of such widely known antibiotics as nistatin and terbinafine (Table 2).

Data reported in Tables 1 and 2 clearly point out that in the cases mentioned above a synergistic effect is present, the antifungal activities of the metal(II) complexes being higher than those of both the ligand and the starting metal salts. This provides reason to believe that antifungal activities of the metal(II) complexes synthesized do not correlate with the toxicity of the metal(II) ions against the fungi tested.

The free ligand and its metal(II) complexes were tested for their anti-HIV activities in cell-based assays (Table 3). To evaluate the inhibitory activity of these compounds the MTC/EC_{50} ratio was used, permitting to judge about the broadness of the antivirus activity range and about the degree of toxicity of the compounds tested. All tests were compared with azidothymidine (AZT) as the positive control carried out at the same time under identical conditions. The data obtained demonstrate a very low ligand activity and its high cytotoxicity, while its complexation with metal(II) ions results in a noticeable activity growth along with a decrease in cytotoxicity, as can be seen from comparing the maximal tolerable concentration (MTC) values for the metal complexes and the ligand (Table 3). It was CuL_2 complex that showed the highest anti-HIV activity among the compounds tested; according to the MTC/EC_{50} value, it may be characterized as a rather active compound, although ranking significantly below AZT. As seen from Table 3, anti-HIV activities of the metal(II) complexes synthesized do not correlate with the toxicity of the metal(II) ions against the viral species tested, as the starting metal salts have demonstrated no anti-HIV activity.

It is noteworthy that antifungal and anti-HIV activities of the compounds examined, by and large, decrease in the sequence: $CuL_2 > CoL_2 \sim NiL_2 > HL$ (Tables 1–3), and their reducing ability declines in the same sequence, as it was shown above. The results of our pilot studies suggest that the metal(II) complexes of sulphurous derivatives of sterically

hindered *o*-diphenols may be of interest in search for novel, more active analogues which could be used in future for designing new chemotherapeutic agents capable both of inhibiting human immunodeficiency virus replication and of exerting antifungal activities.

Acknowledgement

This work was supported by International Science and Technology Center (ISTC grant B-984). We are grateful to Dr. R. Zheldakova (Belarusian State University) for the antifungal testing.

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